

PATENT
10/001,267
Docket 093/004p

CLAIM AMENDMENTS

1 to 12. **CANCELLED**

13. *(Previously presented)* A method for producing differentiated cells from primate pluripotent stem (pPS) cells, comprising:

- a) obtaining a culture of pPS cells;
- b) initiating differentiation of the pPS cells; and simultaneously or subsequently
- c) culturing the cells of step b) in a medium containing ~~a histone deacetylase inhibitor,~~
butyrate until at least ~60% of the cultured cells have at least three of the following characteristics:

- antibody-detectable expression of α_1 -antitrypsin (AAT);
- antibody-detectable expression of albumin;
- absence of antibody-detectable expression of α -fetoprotein;
- RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
- evidence of glycogen storage;
- evidence of cytochrome p450 activity;
- evidence of glucose-6-phosphatase activity; or
- the morphological features of hepatocytes.

14. *(Previously presented)* The method of claim 13, wherein at least about 60% of the cells have at least five of said characteristics.

15. *(Previously presented)* The method of claim 13, wherein at least about 80% of the cells have at least seven of said characteristics.

16 to 18. **CANCELLED**

19. *(Previously presented)* The method of claim 13, wherein differentiation of the pPS cells is initiated by forming embryoid bodies.

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20. *(Previously presented)* The method of claim 13, wherein differentiation of the pPS cells is initiated by culturing in a medium containing dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexmethylene bisacetamide, or another polymethylene bisacetamide.
21. *(Previously presented)* The method of claim 13, comprising further culturing the cells in a medium containing a cytokine or hormone selected from glucocorticoids, epidermal growth factor (EGF), insulin, TGF- α , TGF- β , fibroblast growth factor (FGF), hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.
22. *(Previously presented)* The method of claim 21, wherein the cells are cultured in a medium containing at least three of said cytokines or hormones.
23. *(Previously presented)* The method of claim 22, wherein the cells are cultured in a medium containing EGF, TGF- α , and HGF.
24. *(Previously presented)* The method of claim 13, further comprising maintaining the differentiated cells by culturing them in a medium containing a histone deacetylase inhibitor butyrate.
25. CANCELLED
26. *(Previously presented)* The method of claim 27, wherein the pPS cells are human embryonic stem cells.
27. *(Previously presented)* A method for maintaining hepatocyte lineage cells in culture, comprising:
- obtaining a population of cells differentiated from an established culture of primate pluripotent stem (pPS) cells, wherein at least ~60% of the differentiated cells have at least three of the following characteristics:
 - antibody-detectable expression of α_1 -antitrypsin (AAT);
 - antibody-detectable expression of albumin;
 - absence of antibody-detectable expression of α -fetoprotein;
 - RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
 - evidence of glycogen storage;
 - evidence of cytochrome p450 activity;
 - evidence of glucose-6-phosphatase activity; or
 - the morphological features of hepatocytes; and then
 - culturing the differentiated cells in a medium containing a histone deacetylase inhibitor, butyrate so that at least ~60% of the cultured cells maintain said characteristics.

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28. *(Previously presented)* A method for producing differentiated cells from human embryonic stem (hES) cells, comprising:
- a) obtaining a culture of hES cells;
 - b) initiating differentiation of the hES cells; and simultaneously or subsequently
 - c) culturing the cells of step b) in a medium containing ~~a histone deacetylase inhibitor~~, butyrate until at least ~60% of the cultured cells have at least three of the following characteristics:
 - antibody-detectable expression of α_1 -antitrypsin (AAT);
 - antibody-detectable expression of albumin;
 - absence of antibody-detectable expression of α -fetoprotein;
 - RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
 - evidence of glycogen storage;
 - evidence of cytochrome p450 activity;
 - evidence of glucose-6-phosphatase activity; or
 - the morphological features of hepatocytes.
29. *(Previously presented)* The method of claim 13, wherein the pPS cells are cultured with the ~~histone deacetylase inhibitor~~ butyrate without previously initiating differentiation.
30. *(Previously presented)* The method of claim 13, wherein the pPS cells are cultured on an extracellular matrix without feeder cells before contact with the ~~histone deacetylase inhibitor~~ butyrate.
31. *(Previously presented)* The method of claim 28, wherein at least about 60% of the cells have at least five of said characteristics.
32. *(Previously presented)* The method of claim 28, wherein at least about 80% of the cells have at least seven of said characteristics.
33. **CANCELLED**
34. *(Previously presented)* The method of claim 28, comprising pre-differentiating the cells by culturing in a medium containing dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexamethylene bisacetamide, or another polymethylene bisacetamide.
35. *(Previously presented)* The method of claim 28, comprising further culturing the cells in a medium containing at least three cytokines or hormones selected from glucocorticoids, epidermal

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growth factor (EGF), insulin, TGF- α , TGF- β , fibroblast growth factor (FGF), hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.

36. *(Previously presented)* The method of claim 34, wherein the cells are cultured in a medium containing EGF, TGF- α , and HGF.
37. *(Previously presented)* The method of claim 27, wherein at least about 60% of the cells have at least five of said characteristics.
38. *(Previously presented)* The method of claim 27, wherein at least about 80% of the cells have at least seven of said characteristics.

39-40. CANCELLED

Upon allowance of the application, please renumber the claims as follows:

Claim	13	→	1	Claim	28	→	12
	14	→	2		29	→	4
	15	→	3		30	→	5
	19	→	6		31	→	13
	20	→	7		32	→	14
	21	→	8		34	→	15
	22	→	9		35	→	16
	23	→	10		36	→	17
	24	→	11		37	→	19
	26	→	21		38	→	20
	27	→	18				